

1 **Optimal cultivation length to isolate *Propionibacterium acnes* in suspected bone**
2 **and joint infections is > 7 days**

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13 **Running Head:** Time to positivity in *Propionibacterium acnes*

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18 **ABSTRACT**

19 Diagnosis of *Propionibacterium acnes* bone and joint infection is challenging due to long
20 cultivation time of up to 14 days. We retrospectively studied whether reducing cultivation
21 time to 7 days allows accurate diagnosis without losing sensitivity. We identified patients
22 with at least one positive *P. acnes* sample between 2005 and 2015 and grouped into
23 'infection' and 'no infection'. An 'infection' was defined when at least two samples from
24 the same case were positive. Clinical and microbiological data including time to
25 positivity for different cultivation methods were recorded. We found 70 cases of proven
26 *P. acnes* infection with a significant faster median time to positivity of 6 days (range 2-
27 11 days) compared to 9 days in 47 cases with *P. acnes* identified as a contamination
28 ($p < 0.0001$). In 15 of 70 (21.4%) patients with an infection, tissue samples were positive
29 after day 7 and in 6 patients (8.6%) after day 10 when performing a blind subculture of
30 the thioglycolate broth. Highest sensitivity was detected for thioglycolate broth (66.3%)
31 and best positive predictive values for anaerobic agar plates (96.5%). A prolonged
32 transportation time from the operating theatre to the microbiological laboratory did not
33 influence time to positivity of *P. acnes* growth. By reducing the cultivation time to 7 days,
34 false negative diagnosis would increase by 21.4%, thus we recommend to cultivate
35 biopsies in bone and joint infections to detect *P. acnes* for 10 days with a blind
36 subculture at the end.

37 INTRODUCTION

38 *Propionibacterium acnes* is a facultative anaerobic Gram-positive rod, abundant on the
39 human skin, and mainly associated with sebaceous glands of the shoulder and axilla
40 (1). It is most commonly associated with the chronic skin disease acne vulgaris.
41 However, it may also cause bone and joint infections including implant-associated
42 infections. *P. acnes* has been recognized as an emerging cause of shoulder infections
43 (2, 3) and is among the most common pathogens isolated in shoulder periprosthetic
44 joint infections (PJI) (4, 5). *P. acnes* has also been implicated in other biofilm-related
45 infections (6-8) such as cardiovascular implant-associated infections (9), spinal
46 osteomyelitis (10, 11), and endophthalmitis (12, 13).

47 Diagnosis of *P. acnes* bone and joint infections is challenging since pain is often the
48 only symptom (14, 15). For long-time, *P. acnes* was underdiagnosed in bone and joint
49 infections due to the short cultivation time routinely used in diagnostic laboratories. In
50 general, biofilm forming bacteria are known to replicate at a slow rate due to low
51 metabolism (16). Since recent studies recommended a prolonged cultivation time of up
52 to 14 days for bone and joint infections (17, 18), the diagnosis of *P. acnes* infections has
53 become more frequently documented (19). In view of the high costs of a long incubation
54 period, a recent study suggested that 7 days of incubation should be sufficient for
55 accurately diagnosing orthopedic implant-associated infections (20). In this study,
56 96.6% of the infections were detected within 7 days. However, *P. acnes* caused only
57 one out of the 58 infections. The majority of cases was predominantly caused by
58 *Staphylococcus aureus* and coagulase-negative staphylococci. Other studies attempted
59 to improve diagnostic quality using better cultivation methods or sonication of implants

60 to improve sensitivity and expedite diagnosis (21-24). However, none of these studies
61 included a sufficient number of *P. acnes* infections to perform statistical analysis, which
62 would allow drawing conclusions about the ideal cultivation time for slow-growing
63 microorganisms such as *P. acnes*.

64 This study reports the results of a large cohort of patients with *P. acnes* positive
65 samples, analyzing and comparing time to detection in bone and tissue biopsies, and of
66 sonicated implants with detailed information about transportation time, media types, and
67 handling of specimens. We hypothesized that a higher inoculum as found in bone and
68 joint infections would show growth of *P. acnes* earlier than a lower inoculum due to
69 contamination. Therefore we compared time to positivity in patients with an infection
70 caused by *P. acnes* and isolation of *P. acnes* interpreted as a contaminant. The main
71 aim of the study was to answer the question whether reducing the cultivation time to 7
72 days allows to diagnose *P. acnes*-related bone and joint infections without losing
73 sensitivity.

74 METHODS

75 Study population

76 The orthopedic University Hospital Balgrist in Zurich, Switzerland is a 120-bed
77 orthopedic center specialized for bone and joint infections. Approximately 5000 surgical
78 procedures are performed annually thereof 326 primary hip, 186 knee, and 143
79 shoulder arthroplasties in 2015. In this single-center study, we retrospectively searched
80 for patients with an orthopedic surgery (bone, or joint related) with at least one positive
81 sample for *P. acnes* isolated between January 2005 and December 2015. All positive
82 and negative samples within the same patient, same hospitalization period, and same
83 infection site were grouped as a diagnostic set in a patient case.

84 Microbiological data were searched using the database of the Institute of Medical
85 Microbiology, University of Zurich, Zurich, Switzerland. Clinical and demographic
86 parameters of the patients' medical history at the time of diagnostic work-up including
87 diagnosis were investigated using the patient clinical database of the orthopedic
88 department and the prospective database of the Infectious Diseases Consultation
89 service. We grouped patients into the following 2 groups: 'infection' with growth of *P.*
90 *acnes* in 2 or more samples within the same case and 'no infection' with only 1 sample
91 positive for *P. acnes* suggesting contamination of the sample. For optimal and accurate
92 allocation in one of the two groups 'infection' and 'no infection', only cases with 3 or
93 more samples were included for our analysis (25, 26). Although diagnosis of 'infection'
94 was strictly microbiological defined, we also reviewed clinical presentation of all patients
95 (presence of sinus tract) and investigated the intraoperative presentation. 'Infections'
96 were further grouped as i) associated with a joint arthroplasty (PJI), ii) associated with

97 another orthopedic implant than arthroplasty (implant-associated infections), iii) septic
98 arthritis (often arthroscopy-associated), or as iv) osteomyelitis. Implants other than
99 arthroplasties were labeled as small if screws or anchors were in place, and as large if
100 plates or intramedullary nails were used. Intraoperative samples for microbiological
101 diagnostics could be tissue or bone biopsies (peri- or intraarticular, periimplant) for
102 conventional culture, and the implant itself for culture of sonication fluid in case of
103 removal of the implant (27). In cases with septic arthritis or osteomyelitis only tissue or
104 bone biopsies were investigated.

105 Synovial fluid samples were excluded because we assumed faster time to positivity
106 from recovered planktonic bacteria than biofilm bacteria isolated from periimplant tissue
107 or bone. Samples to analyze should have a minimal acceptable cultivation time in order
108 not to distort statistical analysis. Consequentially we excluded negative samples with a
109 too short cultivation time according to Table 1. Since antibiotic treatment could influence
110 time to positivity of *P. acnes* growth, we excluded samples from patients taken
111 antibiotics for ≥ 24 hours within 14 days prior to sample acquisition. Furthermore, we
112 excluded patients with polymicrobial infections since other bacteria in the same sample
113 - in particular virulent bacteria, could decrease detection of *P. acnes*. All excluded
114 samples and cases are numerated in the Supplementary Table S1. The study was
115 performed in line with the current ethical guidelines and approved by the Institutional
116 Review Board in Zurich, Switzerland.

117

118 **Analysis and statistical methods**

119 For each sample of a patient diagnostic set, we recorded culture details such as type
120 (e.g. tissue, bone, sonication fluid, synovial fluid, or wound swab), culture method, and
121 Gram staining. We calculated time to positivity of each positive sample and culture type
122 in the group of cases with an 'infection' and cases without.

123 Transportation time was calculated as the difference in hours between time of
124 acquisition of samples during surgery and arrival at the microbiological laboratory. Since
125 the exact time of biopsy acquisition had not been recorded in the medical chart, we
126 defined time of acquisition as the mean in time between start and end time of surgery.

127 We analyzed time to positivity, sensitivity, specificity, and positive and negative
128 predictive value for each culture method (direct aerobic, direct anaerobic, and
129 enrichment) for tissue and bone biopsies, and sonication fluid from explanted hardware.
130 To calculate diagnostic sensitivity, we compared test performance in patients with an
131 'infection' (number of positive samples divided by all samples taken within a case) and
132 specificity in patients without (number of negative samples divided by all samples taken
133 within a case).

134 Statistical analysis was conducted using Stata 14.1 SE (StataCorp College
135 Station, Tx). We used non-parametric test-statistics: Wilcoxon rank-sum tests to
136 compare continuous variables and Fisher's exact tests to compare categorical variables.
137 To assess if transportation time from the operation theatre to the microbiological
138 laboratory was correlated with culture time to positivity, we used the Spearman's rank
139 correlation coefficient. Kaplan-Meier curves were used to illustrate the number of days
140 from sampling to culture positivity. For patients belonging to the group of *P. acnes*

141 'infections', time was measured until culture positivity of the second sample to confirm
142 'infection'. Differences between groups were analyzed with log-rank tests.

143

144 **Microbiological processing**

145 *Cultures*

146 **Prediagnostic.** Periimplant-tissue, explanted hardware (implant), and bone samples
147 were transported from the operating room at the orthopedic hospital Balgrist to the
148 microbiology laboratory, which is a 30 to 60 minutes' drive by car. Transport service for
149 microbiological samples is twice daily. Tissue or bone samples were transported in
150 culture tubes without adding transporting media, implants for sonication in ringer lactate
151 solution.

152 **Diagnostic.** To extract bacteria from the tissue, samples were vortexed using 4mm
153 glass beads (Sarstedt, Nümbrecht, Germany). After homogenization, samples were
154 directly incubated under aerobic and anaerobic conditions on agar plates and in aerobic
155 thioglycolate broth (BD, Allschwil, Switzerland) for enrichment. The bone samples were
156 inoculated in aerobic thioglycolate broth only. See Table 1 for cultivation time used in
157 this study. For aerobic cultivation Columbia sheep blood agar without antibiotics
158 (BioMérieux, Mary-l'Etoile, France), Colistin nalidixin acid (CNA) blood agar
159 (BioMérieux), MacConkey agar (BioMérieux), and Crowe agar (chocolate agar
160 supplemented with bacitracin and isovitalex (Difco GC medium, Becton Dickinson))
161 were used. Brucella agar (in house sheep blood agar plates with haemin and vitamin
162 K1, BD), kanamycin-vancomycin agar (laked sheep blood Brucella agar plates with

163 kanamycin and vancomycin, BD), and phenylethylalcohol agar plates (BD) were used
164 for anaerobic cultivation (Whitley anaerobic workstation MG1000, Don Whitley
165 Scientific, West Yorkshire, England). Thioglycolate broth medium was inspected daily
166 for cloudiness, and then subsequently plated onto chocolate (aerob) and Brucella
167 (anaerob) agar plates for further identification. If thioglycolate broth cultures were
168 negative after 10 days of cultivation, blind subcultures plated on chocolate and Brucella
169 agar plates were performed and cultivated for another 2-3 days.

170 Explanted implants were directly placed either in a plastic bag (double bagged) or
171 in a sterile tupper ware container in the operating room and transported to the
172 microbiological laboratory for sonication as previously described using BactoSonic
173 (Berlin, Germany) (28, 29). Under a laminar flow, the future opening sites of the bags
174 were thoroughly disinfected using 70% alcohol and the bags were sliced open using a
175 sterile scalpel. The entire fluid inside the inner bag or the tupper ware container was
176 pipetted into a falcon tube (BD) and inoculated onto the corresponding culture media. Of
177 the sonication fluid, 0.5ml was plated onto the distinct agar plates for aerobic (chocolate
178 and sheep blood agar) and anaerobic (Brucella) cultivation as described for tissue
179 before. 10ml of the liquid was inoculated into blood culture flasks (BacT/Alert FA and
180 FN- bottles (Bio-Mérieux) for aerobic and anaerobic cultivation. The bottles were
181 vertically incubated and slightly shaken at 60 hertz. Flasks with a positive growth- signal
182 were subcultured using the set of agar-based media described above. Cultivation times
183 for different methods are summarized in Table 1. A threshold of ≥ 50 CFU/ml bacteria on
184 direct agar plates was considered as positive.

185 All microorganisms were identified by standard identification methods including
186 MALDI-TOF since 2012. Before 2012, identification of *P. acnes* was performed by
187 conventional reactions such as positive reactions for CAMP, indole, nitrate reduction,
188 catalase, and detection of propionic acid as metabolic fatty acids formed by glucose
189 fermentation.

190 *Microscopy*

191 A Gram stain was performed routinely for tissue samples and sonication fluid from
192 implants parallel to all mentioned methods at day zero in the clinical microbiology
193 laboratory.

194 *Time to positivity of P. acnes growth*

195 We defined time to positivity as the time at which either 1) *P. acnes* typical colonies
196 grew on agar plates, 2) when turbidity in thioglycolate broth, or 3) a positive signal in
197 blood culture bottles were detected for which *P. acnes* was finally identified on agar
198 plates.

199 RESULTS

200 Clinical data

201 We identified 70 cases with a *P. acnes* 'infection', in which 262 out of 379 samples were
202 positive (69.1%). 47 cases did not fulfill our criteria for a proven 'infection' with only one
203 positive sample (47 out of 215 samples, 21.9%). The most common sample site was
204 shoulder (total n = 77), followed by hip (n=26). In the 'infection' group, we diagnosed PJI
205 in 35 (50%) cases, and implant-associated infections with large (plates, intramedullary
206 nail) or small implants (screws, anchors) in 10 (14.3%) and 17 (24.3%), respectively.
207 Five cases presented with septic arthritis (arthroscopy-associated), and three cases
208 with osteomyelitis (two in the shoulder after infiltration or trauma and one in the clavicle
209 associated with b-cell lymphoma). In the 'no infection' group there were pain due to
210 mechanical reasons in 22 (46.8%), aseptic loosening of an implant in 9 (19.2%), and
211 other reasons in 16 (34%) cases diagnosed at revision surgery.

212 There was no significant difference in number of samples taken between the
213 'infection' and 'no infection' group (mean 5.4 and 4.6 samples, respectively, $p=0.06$).
214 The characteristics of cases are shown in Table 2. We found no statistically significant
215 difference with regard to sex, age, or presence of foreign bodies between the two
216 groups. Intraoperative findings described by the surgeon could not help to distinguish
217 between 'infection' and 'no infection'. Isolation of *P. acnes* in the shoulder was more
218 prevalent in the 'infection' group (51 and 26 cases respectively, $p=0.073$) but isolation in
219 the knee was more frequent in the 'no infection' group (1 and 5 cases respectively,
220 $p=0.038$).

221

222 **Time to positivity**

223 The proportion of sample positivity was 55.4% after 7 days and 65.7% after 10 days in
224 the 'infection' group, which was significantly higher than in the 'no infection' group (8.4%
225 and 19.1% respectively, $p < 0.0001$) (Fig. 1).

226 Overall, median time to first positive sample was different in samples of the
227 'infection' group (5 days, 95% CI 4-5) than in the 'no infection' group (9 days, 95% CI 7-
228 10, $p < 0.0001$) (Fig. 2A). Time to confirmation of 'infection', according to our definition
229 equal to time to second positive sample, was also significantly different (6 days, 95% CI
230 5-7) to the single positive sample in the 'no infection' group (9 days, 95% CI 7-10,
231 $p < 0.0001$) (Fig. 2B).

232 Within the 'infection' group, discontinuation of cultures at 7 days would have led
233 to missing diagnosis of 'infection' in 15 out of 70 (21.4%) cases (Fig. 2B). On day 10, a
234 *P. acnes* 'infection' was still not diagnosed in 6 cases (8.6%). These cultures finally
235 turned positive on day 11, all from blind subcultures at the end of regular cultivation of
236 thioglycolate broth. In the 'no infection' group, the majority of cases with a single
237 positive *P. acnes* sample (29 of 47 cases, 61.7%) - suggesting a contamination of the
238 sample - were recorded after day 7 (Fig. 2B).

239 Comparing different cultivation methods (Table 3) in the 'infection' and 'no
240 infection' group, we found that thioglycolate broth of tissue biopsies showed a significant
241 difference in median time to positivity (6 days, 95% CI 5-6 and 9 days, 95% CI 8-10;
242 $p < 0.0001$) as opposed to other methods, which did not significantly differ.

243

244 **Sensitivity, specificity, positive and negative predictive value**

245 Thioglycolate broth as the enrichment method used for tissue and bone biopsies was
246 most effective in correctly identifying *P. acnes* (sensitivity 66.3% in tissue and 75% in
247 bone) and significantly different to aerobic and anaerobic agar plates (sensitivity 5.1%
248 and 42.1% respectively; p-value 0.0001). Low sensitivity was observed in all aerobic
249 cultures (direct aerobic agar or aerobic blood culture bottles) (Table 4). Thioglycolate
250 broth as the method with the highest sensitivity, however, showed a low specificity in
251 tissue (79.1%) and bone (66.7%).

252 Best specificity with low false-positive results was observed when aerobic agar or
253 aerobic blood culture bottles were used as the enrichment method. Among tissue and
254 sonication fluid cultures, both direct anaerobic cultivation methods showed the best
255 positive predictive value with 96.5% and 86.7% respectively (Table 4).

256 **Gram staining**

257 Only 10 out of 311 (3.2%) samples in the group of patients with a bone and joint
258 'infection' with an available Gram staining showed a positive result with Gram-positive
259 rods. No positive Gram staining was observed in any of the samples from 'non-infected'
260 individuals resulting in a 100% positive predictive value and 36.9% negative predictive
261 value.

262 **Influence of transportation time on diagnostic output**

263 Transportation time was available in 528 of 594 samples (88.9%) out of which 336
264 samples were allocated to the 'infection' group and 192 to the 'no infection' group.
265 Transportation time of less than 24 hours was seen in 94% of the positive samples in

266 the 'infection' group and in 92% in the 'no infection' group. Transportation time did not
267 correlate with time to positivity (Spearman's $\rho = -0.0195$, $p = 0.66$).

268 **Sub analysis of 35 cases with a PJI caused by *P. acnes***

269 In a sub analysis of 35 cases with a PJI caused by *P. acnes*, we compared diagnostic
270 sensitivity of sonication fluid with tissue biopsy cultures. Only 26 cases could be
271 included for this sub analysis with both sonication fluid and tissue biopsy cultures
272 available. 8 cases could not be included due to only having tissue samples and 1 case
273 due to only having tissue and bone samples analyzed.

274 For tissue biopsies, the sensitivity was 96.2% (25/26 cases) with at least 1 positive
275 culture as opposed to sonication fluid with 46.2% (12/26). 23 cases had ≥ 2 positive
276 tissue samples, 2 had only 1, and 1 had no positive tissue at all. All 3 cases (11.5%)
277 with < 2 positive samples had a positive sonication > 100 cfu/ml to be classified as an
278 'infection' and would have been misclassified as a contaminant if only tissue biopsies
279 had been cultivated.

280 **DISCUSSION**

281 In this large cohort-study of 70 *P. acnes* 'infections', we showed that median time to
282 positivity to confirm an 'infection' was 6 days with a range up to 11 days. However,
283 reducing cultivation time to 7 days as proposed by previous studies (20, 23) would have
284 resulted in missing diagnosis in 15 patients (21.4%) suggesting that in patients with a
285 high prevalence of *P. acnes* infections a prolonged cultivation is crucial for detecting
286 infections. This supports the results of a study including fewer *P. acnes* infections (37
287 cases), which showed that 14% of positive samples were detected after day 7 (3).
288 Furthermore, the study showed that a prolonged cultivation time of > 10 days did not
289 improve sensitivity. Therefore Frangiamore et al. recommended that 10 days of routine
290 cultivation time is optimal to assure accurate diagnosis of *P. acnes* infections. Our data
291 support this recommendation; However, we would recommend a 10-day cultivation with
292 a blind subculture from the thioglycolate at the end in certain cases with high suspicion
293 of *P. acnes* infection. We would have missed 6 cases (8.6%) if we had stopped cultures
294 at day 10.

295 We reported median time to first positive sample of 5 days. A study by Minassian
296 et al. using blood culture bottles as an enrichment method for tissue biopsies, showed
297 similar results (median time to positivity of 5 days, range 3-13 days) but it was of smaller
298 size with 30 *Propionibacterium* sp. isolates from 16 patients. Butler-Wu et al. described
299 the mean time to positivity of 7.3 ± 2.6 days with a range of up to 13 days for 19 infected
300 cases undergoing revision arthroplasty (18). *P. acnes* grew significantly faster in
301 samples isolated from patients with a *P. acnes* 'infection' as compared to patients
302 without an 'infection'. This difference was most pronounced in enrichment thioglycolate

303 broth supporting previous studies (3) and our hypothesis that a higher inoculum is to be
304 found in infected samples as compared to contaminated samples.

305 Thioglycolate broth enrichment during 10 days is a sensitive method to detect *P.*
306 *acnes*. However, a prolonged incubation time also increases the risk of cultivating a
307 bacterial contamination (30, 31) as shown by our result that 61.7% of samples
308 belonging to our 'no infection' cases were recorded after day 7. Our results are
309 consistent with those of a previous study showing that 21.7% of the cases with only 1
310 positive *P. acnes* sample - labeled as 'no infection' - became positive after day 13 (18).
311 A previous study published 2013 showed a faster time to positivity when thioglycolate
312 broth was cultivated anaerobically (30) which could be investigated in a prospective
313 study in our orthopedic center. Avoiding the problem of low specificity, we found the
314 best positive predictive value was for tissue biopsies grown directly on anaerobic agar.
315 All aerobic cultivation methods showed low sensitivity, which is not surprising since *P.*
316 *acnes* is facultative anaerobic. We did not find an increased sensitivity using both
317 aerobic and anaerobic cultures as Butler-Wu et al. published (18).

318 Gram staining is not a useful tool for excluding bone and joint infections. All of
319 our 10 positive Gram stains were from 'infected' cases. This supports published data
320 showing that positive results prove infection while a negative result does not exclude an
321 infection (32, 33).

322 Cultivation of sonication fluid of implants is known to show a better sensitivity
323 than conventional tissue cultures (27). This is also shown in a recent study by Portillo et
324 al. who investigated sensitivity of sonication in 39 orthopedic implant-associated
325 infections including 5 cases with a *P. acnes* infection. They detected all 5 *P. acnes*

326 infections by sonication but only 2 by conventional tissue cultures (22). A retrospective
327 cohort study of 20 PJIs caused by *P. acnes* showed an 89% sensitivity for sonication
328 fluid cultures and 60% for conventional tissue cultures (15). Our results of lower
329 sensitivity in sonication than in tissue cultures refute this observation: The most obvious
330 explanation for our findings is our short cultivation time of only 3 days on agar plates
331 and 7 days in blood culture bottles compared to a longer cultivation time in tissue or
332 bone biopsy cultures. Other studies using cultivation times of up to 14 days in blood
333 culture bottles reported a high sensitivity for sonication (21, 22, 24). An alternative
334 explanation for our low sensitivity with sonication is the possibility that *P. acnes* might
335 be inhibited or killed using ultrasound baths as has been shown for other pathogens
336 (34). Since we had initially used plastic bags instead of tupper ware containers for
337 sonication for many years (using a higher ultrasound frequency of 45kHz), this could
338 contribute to the difference and further emphasizes the necessity to adhere to a
339 standard protocol. Our results of better sensitivity of direct agar planting for 2-3 days
340 compared to cultivation using blood culture bottles for 7 days is intriguing particularly in
341 light of the increasing number of studies that point to improved performance of blood
342 culture media for the recovery of pathogens causing PJI (21, 22, 24). An advantage of
343 using blood culture bottles instead of thioglycolate broth medium with presence of
344 antibiotic absorbing agents could not be assessed at this point since we excluded
345 patients taking antibiotics.

346 We showed that a longer transportation time from the operating theatre to the
347 microbiology laboratory did not influence time to positivity of *P. acnes* growth. This is in
348 contrast to recent IDSA recommendation (35) emphasizing that anaerobic tissue

349 cultures should be transported at room temperature in anaerobic containers as soon as
350 possible with an optimal time of 2 hours. However, our result supports the hypothesis
351 that microorganism in the biofilm state as found in bone and joint infections are robust to
352 environmental changes in particular *P. acnes* as a facultative anaerobic bacterium. We
353 could not find another study investigating the influence of time of transportation or use
354 of anaerobic transport container for recovery of *P. acnes* in bone and joint infections.

355 The strength of our study is the large cohort of 70 cases with a *P. acnes*
356 infection. To our knowledge, this is the largest study to date with analysis of the applied
357 cultivation methods and time to positivity in bone and joint infections focusing on *P.*
358 *acnes* as a slow growing microorganism. Most study results in this field are influenced
359 by various variables. To strengthen the conclusions of our analysis, we excluded
360 samples obtained from individuals already on antibiotics as well as cases with a
361 polymicrobial infection. The Strength of our study is the time period of over 10 years in
362 which the same microbiological protocols were used for all bone and joint infection
363 samples. A limitation of our study is that different culture media were not incubated for
364 the same period of time, which makes comparison difficult but due to the retrospective
365 study design difficult to correct.

366 We conclude that a prolonged cultivation time of 10 days is necessary for *P. acnes*
367 identification. We do not recommend reducing cultivation time to 7 days in patient
368 cohorts with a high incidence of *P. acnes* infections (e.g shoulder PJI, vertebral
369 osteomyelitis). We would have missed 21.4% of the *P. acnes* infections if cultivation
370 time had been reduced to 7 days. Thioglycolate broth as an enrichment method in
371 tissue biopsies showed a high sensitivity. The best positive predictive value was seen

372 with direct incubation on anaerobic agar plates. Time to positivity of *P. acnes* growth
373 does not seem to be affected by a prolonged transportation time, which shows that *P.*
374 *acnes* in the biofilm of musculoskeletal infections can survive for hours even in a
375 fastidious environment.

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498

499 **TABLES AND FIGURES**

500 **Table 1.** Standard cultivation times for periimplant-tissue, implant (sonication fluid), and
 501 bone and accepted minimum cultivation time to be included in our study.

	Cultivation method	Standard cultivation time (days)	Accepted cultivation time for inclusion (days)
Tissue	Direct aerobic	7	≥6
	Direct anaerobic	7	≥6
	Thioglycolate aerobic	10 (-14*)	≥9
Implant	Direct aerobic	2-3	≥2
	Direct anaerobic	2-3	≥2
	Aerobic BCB	7	≥6
	Anaerobic BCB	7	≥6
Bone	Thioglycolate aerobic	10 (-14*)	≥9

502 *prolonged culture with blind subculture at the end

503 BCB, blood culture bottles

504

505 **Table 2.** Characteristics of 70 cases with a bone and joint 'infection' (≥ 2 positives *P.*
506 *acnes* samples) and 47 cases with 'no infection' (1 positive *P. acnes* culture).

	'Infection' (n =70)	'No infection' (n =47)	p-value
Patient characteristics			
Sex, female (%)	21 (30%)	15 (32%)	0.84
Age years, median (range)	58 (21-81)	58 (16-86)	0.49
Sample site n (%)			
Shoulder	51 (72.9%)	26 (55.3%)	0.07
Hip	13 (18.6%)	13 (27.7%)	0.27
Spine	4 (5.7%)	1 (2.1%)	0.65
Knee	1 (1.4%)	5 (10.6%)	0.04
Other	1 (1.4%)	2 (4.3%)	0.56
Presence of			
any foreign body n (%)	62 (88.6%)	39 (83%)	0.42
prosthesis n (%)	35 (50%)	27 (57.4%)	0.46
Intraoperative presentation			
Normal	7 (10%)	6 (12.8%)	0.77
Pus	9 (12.9%)	2 (4.3%)	0.20
Inflammation	41 (58.6%)	26 (55.3%)	0.85
Wear of the implant	1 (1.4%)	2 (4.3%)	0.56
Adhesion	10 (14.3%)	7 (14.9%)	1

	'Infection' (n =70)	'No infection' (n =47)	<i>p</i> -value
No data available	2 (2.8%)	4 (8.5%)	0.22

507 Nr, number; SD, standard deviation

508

509 **Table 3.** Median time to positivity (TtP) for different cultivation methods in the 'infection'
510 and 'no infection' group.

Culture type	Culture method	'Infection'		'No infection'	
		Positive cultures (Nr)	TtP, days (median, 95% CI)	Positive cultures (Nr)	TtP, days (median, 95% CI)
Tissue	Direct aerobic	15	7 (5-7)	2	7
	Direct anaerobic	136	6 (6-6)	5	6
	Thioglycolate aerobic	205	6 (5-6)	38	9 (8-10)
Implant	Direct aerobic		-		-
	Direct anaerobic	13	4	2	3
	Aerobic BCB		-		-
	Anaerobic BCB	4	4	1	5
Bone	Thioglycolate aerobic	6	7	2	8

511 Nr, number; BCB, blood culture bottles; CI, confidence interval; TdP, Time to Positivity

512 **Table 4.** Sensitivity, specificity, positive predictive value (PPV), and negative predictive
513 value (NPV) of different culture methods

	Culture method	Sensitivity	Specificity	PPV	NPV
Tissue	Direct aerobic	5.1%	98.9%	88.2%	38.8%
	Direct anaerobic	42.1%	97.1%	96.5%	47.3%
	Thioglycolate aerobic	66.3%	79.1%	84.4%	58.1%
Implant	Direct aerobic	0%	100%	0%	36.4%
	Direct anaerobic	37.1%	91.3%	86.7%	48.8%
	Aerobic BCB	0%	100%	0%	38.3%
	Anaerobic BCB	13.3%	95.2%	80.0%	43.5%
Bone	Thioglycolate aerobic	75.0%	66.7%	75.0%	66.7%

514 PPV, positive predictive value; NPV, negative predictive value

515 BCB, blood culture bottles

516 **FIGURE LEGENDS**

517 **Fig. 1.** Proportion of sample positivity with *P. acnes* in the 'infection' and 'no infection' group. The colored areas represent
518 the 95% confidence interval. The proportion of positivity at day 7 was 55.4% and 65.7% at day 10 in the 'infection' group.
519 In the 'no infection' group only 8.4% were positive at day 7, and 19.1% at day 10 ($p < 0.0001$). Discontinuation of sample
520 cultivation would have resulted in missing 52 of 262 (19.9%) positive samples or 15 of 70 (21.4%) cases.

521 **Fig. 2.** Proportion of first (A) or second (confirmation) (B) positive sample in the 'infection' group compared to the single
522 positive sample in the 'no infection' group. The colored areas represent the 95% confidence interval. The median time to
523 positivity was 5 (95% CI 4-5) days for the first sample, 6 (95% CI 5-7) days for the second positive sample in the 'infection'
524 group to confirm 'infection', and 9 (95% CI 7-10) days in the 'no infection' group.

525





